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On the Mechanism of Protein Synthesis

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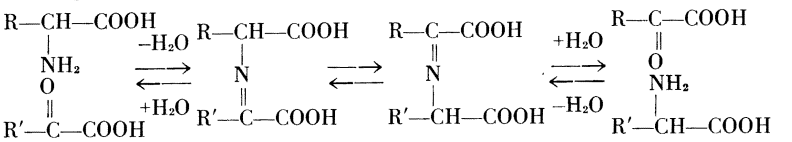
Our knowledge about the mechanism of protein synthesis, one of the most important biochemical problems, is still extremely limited. It is evident from the very nature of proteins, their complexity and specificity, that the problem of protein synthesis presents numerous aspects. Theories of a template method of protein synthesis in the sense of Langmuir-Schaefer or Pauling are designed to explain the specificity of proteins. This paper will deal with only one aspect of protein synthesis, the mode of formation of the amide (peptide) linkage, which is the most typical linkage of every protein. In view of the close connection between protein synthesis and growth, any advance in understanding the former can be expected to shed some light not only on normal growth but also on abnormal growth as typified by cancer formation. It is therefore essential that ideas on the problem of protein synthesis be communicated whereby impetus will be given for research efforts along new avenues of attack. On the more different fronts this problem will be tackled, the greater is the chance of ultimately solving it.

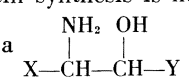
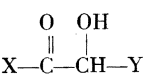
It is well known that strenuous conditions are required for the reaction of a carboxylic acid with an amino compound to yield the amide and water. Pyrolysis of the salt first formed by the two reactants is the usual procedure. Similarly, the self-condensation of amino acids by chemical means requires highly elevated temperatures and is frequently accompanied by extensive decarboxylation. By contrast, protein synthesis in biological systems proceeds at a relatively low temperature and in the presence of water. It is generally recognized, of course, that in such systems other substances are present which may be intimately connected with the reaction. These substances may have a catalytic effect on protein synthesis (such catalysis is not necessarily of a purely physical character, but may, to a large extent, be of a chemical nature), or some other metabolic change may be coupled with the formation of proteins. Our approach is to find an explanation for the formation of the amide linkage under biological conditions in terms of chemical reactions.

Carboxylic acid esters, provided that they possess some water solubility, are known to react with aqueous ammonia or amine solution at ordinary temperature with formation of amides and alcohol. Thus, on reaction with aqueous ammonia at room temperature ethyl acetate yields acetamide and ethyl alcohol; similarly, γ-butyrolactone

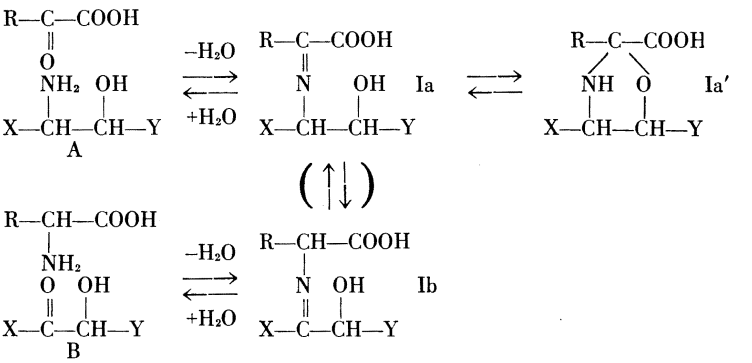
(a cyclic ester) yields γ-hydroxybutyramide. Esters of amino acids are capable of undergoing self-condensation at room temperature. Interestingly enough, such self-condensations proceed far more rapidly in the presence of moisture or in aqueous solution than with the pure esters or their solutions in anhydrous solvents (1). The cyclic diketopiperazines (there are two amide linkages in each diketopiperazine molecule) are the usual products. It has been found, however, that when the self-condensation is caused to take place at a very slow rate by the use of appropriate conditions, chiefly by the exclusion of moisture, appreciable quantities of polypeptide esters, e.g. $H_2NCH_2CO(NHCH_2CO)_nNHCH_2COOC_2H_5$ from $H_2NCH_2COOC_2H_5$, are produced in addition to the diketopiperazine (2,3). Whichever products are obtained, the fundamental reaction is amide formation. Steric circumstances favor the formation of a diketopiperazine when two molecules of amino acid ester react. It is to be expected that interaction between an amino acid ester and the terminal amino group of a polypeptide will result in the lengthening of the polypeptide chain by one amino acid unit; water, if present, could merely affect the speed of this reaction, not its course. We must therefore seriously consider the possibility that amino acids must be esterified before they can be joined to a protein molecule. In the living organism such amino acid esters may well be glycerides. Glycerides of amino acids or glycerides containing both fatty acid and amino acid groups in the same molecule have never been isolated from biological systems, but failure to detect them cannot be considered as evidence against their existence, since they may be highly reactive intermediates with resultant low concentration.

I wish to outline an alternative scheme in which cyclic esters derived from amino acids act as intermediates in protein synthesis. First, it should be pointed out that by the familiar process of transamination probably every α-amino acid can be transformed into the corresponding α-keto acid and vice versa.

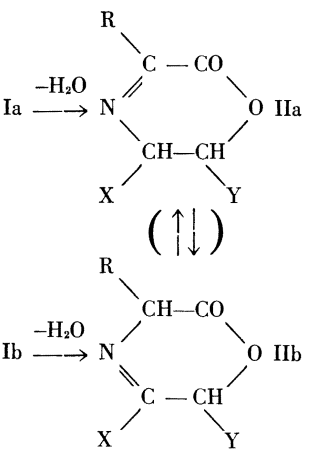


A special role in protein synthesis is now assigned to substances having the general formula  (type A) and/or the general formula  (type B). As will be pointed out later

in more detail, many such substances occur in biological systems. Reaction of type A with an α-keto acid can be expected to give rise to imine Ia, while reaction of type B with an α-amino acid would yield imine Ib. An oxazolidine structure Ia′ might well be in equilibrium with Ia. In some instances imines Ia and Ib will be interconvertible by a tautomeric shift. This will certainly be the case if X=COOH, i.e. if type A is an α-amino-β-hydroxy acid, since then the graph below becomes a special case of the transamination reaction previously shown.



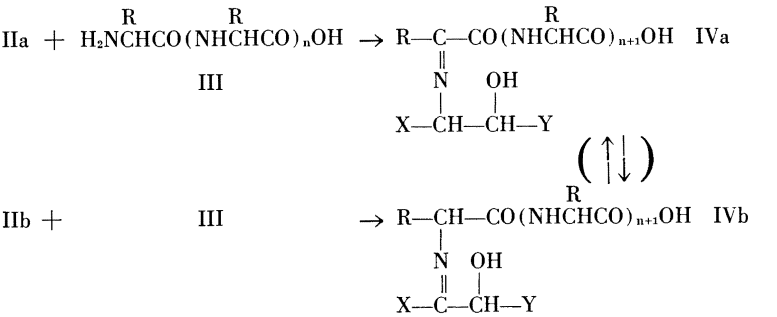
Consideration of the structures of imines Ia and Ib is interesting. Both these compounds are δ-hydroxy acids and it appears likely that they should be able to lactonize with elimination of water to the δ-lactones IIa and IIb respectively. The two lactones will be interconvertible by tautomerism in certain cases, e.g. if X=COOH.



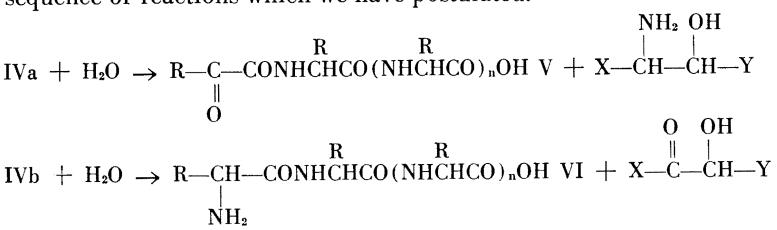
While in most cases δ-hydroxy acids do not lactonize as readily as γ-hydroxy acids and δ-lactones are less resistant to hydrolysis than γ-lactones, a literature survey has shown that δ-hydroxy acids with

a double bond in the α-β or β-γ positions lactonize with great ease, provided that the configuration of the double bond is such that the alcohol and acid portions of the molecule are cis with respect to each other; furthermore, the resulting α-β or β-γ unsaturated δ-lactones are very stable towards hydrolysis. It will be noticed that Ia and Ib have an unsaturation (albeit an imino double bond) in the α-β and β-γ positions respectively. Both Ia and Ib can be expected to be present, at least to a considerable extent, in the cis-configuration required for lactone formation, as cis $\rightleftarrows$ trans isomerization would be easily accomplished by hydrolysis and reformation of the imine involved.

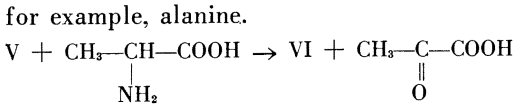
Reaction of IIa, in its capacity as a cyclic ester, with the amino group of an amino acid (III, n = 0) or that of a polypeptide or protein (III, n = 1, 2, 3$\cdots$) would yield IVa. In a similar fashion IIb would give IVb. In these reactions a new amide linkage is created.



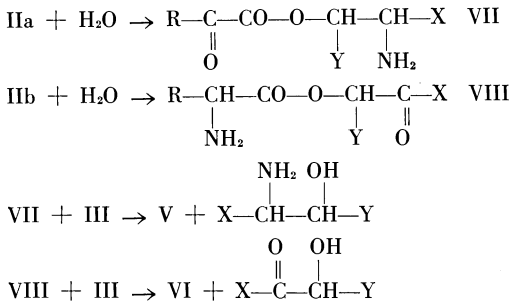
Hydrolysis of IVa would result in V and the type A substance, that of IVb would yield VI and the type B substance. The type A or B substance liberated at this stage may, of course, become involved in the synthesis of a new amide linkage by a renewal of the sequence of reactions which we have postulated.



VI has a free amino group and can function in the manner of III. V could be changed to VI by a transamination reaction involving, for example, alanine.

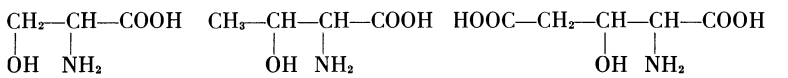


It is possible, of course, that the lactones IIa and IIb are first hydrolyzed at the imino linkage to give VII and VIII respectively. Ultimately, however, the same products as above would result.

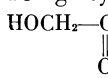


It is a consequence of our scheme that protein synthesis must proceed by the repeated addition of single amino acid units and not by the piecing together of larger building stones of a polypeptide nature, so-called plasteins. This follows from the necessity of having a free amino or keto group in a position alpha to the carboxy group which is about to engage in the formation of an amide linkage. A further consequence would be that a polypeptide structure or near-protein can undergo addition of a new amino acid unit only at an amino group and not at a carboxy group. The interesting experimental work of Geiger (4) provides evidence against the plastein theory of protein formation, which, as we have pointed out, is excluded by our reaction scheme.

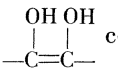
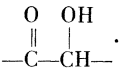
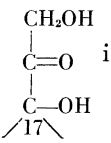
We shall now consider some substances of type A and B known to be present in biological systems. The type A structure is incorporated into a variety of amino acids.



Serine Threonine Hydroxyglutamic acid

Threonine, incidentally, is a dietary essential amino acid for humans. By transamination the corresponding type B compounds i.e. the α-keto-β-hydroxy acids can be obtained in the organism. It is very likely that the precursor of serine, an amino acid capable of being synthesized in the human body, is hydroxypyruvic acid  , the type B analog of serine. Hydroxypyruvic acid is probably an oxidation product of glycerol, glyceraldehyde, or glyceric acid, all products of the carbohydrate metabolism, and is transformable into serine by transamination.

Noradrenaline and norephedrine, likely precursors of adrenaline and ephedrine respectively, also have the type A structure. The simplest type A compound is 2-aminoethanol, which has considerable biological significance.

The type B structure is present in glyceraldehyde, in the aldehydo or keto forms of reducing carbohydrates, and in ascorbic acid (Vitamin C). The enediol grouping  commonly written for a portion of the ascorbic acid molecule is tautomeric with the ketol grouping  . Many steroids also possess the type B structure. Most hormones of the adrenal cortex have the $-COCH_2OH$ side-chain attached to C 17, and in a number of them that same C bears, in addition, a hydroxy group. Thus the grouping  is present in 17-hydroxycorticosterone (Kendall's Compound F) and 17-hydroxy-11-dehydrocorticosterone (Kendall's Compound E), both of which have attained great prominence recently.

No attempt is made here to list all biologically important substances with type A or B structures. It is realized that only one or a few substances of type A or B may actually play the role in protein synthesis which we have outlined. The concentration of such a substance or substances in any one part of the living organism or, for that matter, in any one cell must be very carefully controlled by the organism and intimately connected with its metabolism, if the synthesis of proteins is to take place in a manner that takes account of the structural and functional needs of the organism. A disturbance of this highly sensitive system of controls might well be responsible for a cancerous development.

The importance of the related problem of protein hydrolysis is, of course, recognized. Whether the process can take place by steps that are the exact reverse of those here suggested for protein synthesis or whether some other mechanism must be operative is impossible to say.

Experiments of a chemical as well as of a biological nature de-

signed to put these ideas to test seem worthwhile in view of the importance of the problem of protein synthesis.

References

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2. Curtius, T., Ber., *37*, 1285 (1904).
3. Frankel, M., and Katchalski, E., J. Am. Chem. Soc., *64*, 2264 and 2268 (1942).
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